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Bone Marrow Micrometastases in a Patient With Localized Wilms' Tumor

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The case of a 7-year-old boy presenting at diagnosis with a localized (stage III) Wilms' tumor of favorable histology is presented. Immunocytologic analysis of bone marrow aspirates revealed cells positive for neural cell adhesion molecule (NCAM) and negative for class I major histocompatibility complex (MHC) antigens. These cells were interpreted as deriving from the tumor blastemal component. Postoperatively the child underwent radiotherapy and chemotherapy, and he remains free of disease 12 months after completion of therapy. In patients with nonmetastatic Wilms' tumor at onset, the evaluation of the actual frequency of occult marrow involvement and the assessment of its clinical significance may necessitate further investigation.

Key words: Wilms' tumor, minimal bone marrow disease, neural cell adhesion molecule (NCAM), class I major histocompatibility complex (MHC)

INTRODUCTION

Wilms' tumor presents with overt distant metastases in only 12% of patients at diagnosis, the main metastatic sites being lungs (93%), liver (15%), and distant lymph nodes (10%) [1]. The striking rarity of bone metastases detectable in 0.5–3% of cases with disseminated disease at diagnosis [1–4] has not prompted an extensive bone marrow assessment as part of the diagnostic workup for clinical staging. To our knowledge, cytologic evidence of bone marrow infiltration not associated with local radiologically detectable osteolysis has been previously reported only in one Wilms' tumor patient with metastatic disease [5]. Even less is known about the possibility of cytologic detection of marrow involvement in nonmetastatic cases.

Furthermore, standard cytologic examination of bone marrow aspirates may not be sensitive enough to detect single tumor cells or oligocellular micrometastases. More sensitive and specific immunocytologic testing is needed to reveal this minimal (or occult) marrow involvement that is probably more common than expected from mere clinical findings, even in apparently localized disease [6]. Occult marrow micrometastases, in fact, have been detected at diagnosis in neuroblastoma [7], breast cancer [8–10], and colorectal cancer [11,12], where their presence has been shown to be associated with a high risk for recurrence. At the present, however, there are no reports of minimal bone marrow disease in patients with Wilms' tumor.

We describe the case of a child with nonmetastatic Wilms' tumor of favorable histology in which immunocytologic analysis at diagnosis revealed bone marrow involvement in contrast to the negative findings obtained with standard cytology.

CASE REPORT

A 7-year-old boy presented to our department with an abdominal mass in the right hypochondrium. Ultrasonography revealed a solid nonhomogeneous pararenal mass measuring 15 × 11 × 10 cm. Abdominal CT scans with contrast medium showed normal elimination by both kidneys and no infiltration of neighboring structures. Chest X-rays and CT scans were negative. X-ray skeletal survey did not demonstrate metastatic lesions. Urinary levels of homovanillic acid (HVA) and vanillylmandelic acid (VMA) were normal. Nephrectomy was performed and five regional lymph nodes were removed. Immediately before surgery four bone marrow aspirates were also obtained from the iliac crests.

Macroscopic examination of the surgical specimen confirmed the presence of a mass arising from the kidney and whose measurements were 14 × 11 × 9.5 cm. Microscopic analysis revealed a triphasic Wilms' tumor of favorable histology according to Beckwith [13]. Two

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lymph nodes were infiltrated. The tumor was classified as stage III according to the National Wilms' Tumor Study (NWTS) staging system [14], and the patient then underwent radiotherapy and chemotherapy according to the Nephroblastoma Clinical Trial No. 9 of the International Society of Paediatric Oncology (S.I.O.P.) [15]. Bone marrow aspirates were repeated immediately following chemotherapy. Twelve months after treatment completion, the child remains free of disease.

Bone marrow assessment was carried out both with standard cytology and immunocytology. Cytologic examination was done on at least three smears per aspirate using May-Grunwald-Giemsa stain. Immunocytologic analysis was performed by immunofluorescence according to a modified version of the double-labeling method exploiting the lack of expression of class I major histocompatibility complex (MHC) antigens on the surface of neuroblastoma tumor cells [16]. The use of a monoclonal antibody (MoAb) that recognizes target cells (but cross-reacts with some marrow cells) combined with an anti-class I MoAb that detects only marrow cells, allows for a definite identification of single-labeled target cells.

Briefly, bone marrow samples were diluted with Hanks' balanced salt solution containing 2% fetal-calf serum (FCS), layered over Ficoll-Paque (Pharmacia LKB Biotechnology, Uppsala, Sweden) and separated by density gradient centrifugation at 600 × g for 15 minutes. Mononuclear cells (MNCs) were collected, washed twice in phosphate-buffered saline (PBS) with 2% FCS, and checked for viability using the trypan blue dye exclusion test. Approximately 5.0×10^5 MNCs were incubated with UJ13A MoAb (kindly provided by Dr. J.T. Kemshead, Imperial Cancer Research Fund, Bristol, UK) [17] for 30 minutes at room temperature, washed twice, and then incubated with rhodamine-labeled $F(ab)_2$ goat antimouse immunoglobulin for 30 minutes at 4°C. After washing, MNCs were incubated with a fluorescein-conjugated anticlass I MoAb (Becton Dickinson, Mountain View, CA) for an additional 30 minutes at 4°C. Following two washes, the bound antibodies were visualized using a Zeiss fluorescence microscope with epi-illumination optics. Three separate visual counts were performed, each of which included >1,000 cells. Positive controls (single-labeled for rhodamine) were cultured neuroblastoma cell lines, whereas negative controls (single-labeled for fluorescein or, rarely, double-labeled for both fluorochromes) were normal marrow cells.

The cytologic examinations of all the aspirates were negative. Nevertheless, immunocytologic analysis before chemotherapy showed positive staining for tumor cells in 4% of the examined mononuclear cells, whereas after chemotherapy no positive cells were found.

DISCUSSION

Minimal bone marrow involvement in nonmetastatic Wilms' tumor has not yet been reported. One reason is probably because the workup for this tumor does not routinely require marrow assessment either with standard cytology or immunocytology. However, this does not necessarily mean that tumor cells are not present but simply that they might not reach the detection threshold or ever will during the clinical course. For instance, in the case reported here, cytologic examination of the bone marrow was always negative both before and after chemotherapy, whereas tumor cells were demonstrated to be present only with a more sensitive immunocytologic technique and only before chemotherapy. Likewise, in other solid tumors occult marrow involvement has been detected using immunocytochemical or molecular techniques even in morphologically normal samples [6].

In this case, positive immunostaining was obtained for UJ13A MoAb whose binding to primary Wilms' tumor tissue has previously been described [17,18]. This MoAb in fact recognizes the neural cell adhesion molecule (NCAM) [18], a member of the immunoglobulin superfamily of cell adhesion molecules [19,20] expressed not only by the majority of neuroectodermal tissues [21] but also by the embryonic kidney [22,23] as well as by Wilms' tumor, both in continuous cell lines [24] and in primary tumors [23,25]. Previous studies have demonstrated that among the three different components of this tumor—blastema, epithelium, and stroma—NCAM expression is mainly detected in the blastema and to a lesser extent in the epithelium [23,25,26]. Moreover, since UJ13A MoAb may cross-react with <1% of normal marrow cells [27], the possibility of false positives was ruled out in this case by using a double-labeling method that discriminates marrow cells from tumor cells on the basis of their differential class I MHC antigen expression [16]. Both the epithelial and stromal components have been shown to express class I antigens, whereas the blastemal component has not [28]. Therefore, the immunophenotype of the tumor cells detected in the bone marrow (NCAM positive and class I negative) supports their origin from the blastemal component.

Questions arise as to the biologic and clinical significance of occult Wilms' tumor cells in bone marrow. It is known that to produce a metastasis, a tumor cell must complete a series of sequential and selective interactions with different homeostatic factors of the host [29]. Thus before hematogenous metastases can occur, tumor cells must first enter the bloodstream, i.e., intravasation must occur, and this step is probably more frequent than expected. For instance, in patients undergoing nephrectomy for renal cell carcinoma, more than 1×10^6 tumor cells have been estimated to be released daily into the renal vein [30]. However, due to the low survival rate of tumor cells during the post-intravasation phases, neither the presence of circulating cells nor their arrest within the microcirculation is a guarantee that a metastasis will actually occur. Studies using experimental animal models have demonstrated that radiolabeled tumor cells, al-

though arrested in many organs, survive and proliferate to form metastases only in a few appropriate sites [31,32]. Therefore, even though the number of circulating tumor cells can be high, the estimated rate of metastasis is extremely low, on the order of 1 in 10^5 or 10^6 cells [33]. This is what has been defined as "metastatic inefficiency" [33,34].

Regarding the probability of detecting marrow involvement in Wilms' tumor patients, data concerning the number of possible circulating tumor cells are not yet available. However, since some marrow stromal cells are positive for NCAM [35] and since NCAM can mediate intercellular adhesion by homotypic (NCAM to NCAM) binding [19,36], the NCAM-expressing stromal cells could serve as a "meshwork" recognizing and concentrating those rare circulating tumor cells. The marrow microenvironment may thus serve as a "sorter" for malignant cells shed into the bloodstream and may represent a favorable and accessible site for their search in Wilms' tumor. Notwithstanding this possibility, the rarity of bone lesions in Wilms' tumor [1–4] strongly suggests that the marrow microenvironment must be inadequate for subsequent tumor growth. Tumor cells here entrapped, however, could represent an indicator for a metastatic capability that can be expressed only where an adequate microenvironment exists, e.g., in the lungs.

The finding of marrow involvement at diagnosis in an apparently localized tumor is not so surprising, since occult marrow micrometastases at the time of initial surgery also may be detected with immunocytochemistry in other nonmetastatic solid tumors such as neuroblastoma [7], breast cancer [8–10], and colorectal cancer [11,12]. Even taking into account the likely low metastatic efficiency of these cells, it is reasonable to assume that the higher their number, the greater the probability that one of these might have already acquired the metastatic phenotype. Accordingly, increasing evidence shows that in the above-mentioned tumors, the presence at diagnosis of occult marrow micrometastases is an indicator of poor outcome identifying subsets of patients with apparently localized tumors but at high risk for recurrence [7–12].

Despite the excellent outcome in children with nonmetastatic Wilms' tumor of favorable histology, recurrences and deaths still occur [14]. Prognostic factors, such as age at diagnosis, tumor size, and regional lymph node involvement, already have been established [37]. It has been suggested that these are not per se responsible for the poor outcome but rather may be indicative of a higher probability of micrometastases seeded before diagnosis and not ablated by chemotherapy [37]. In particular, as far as regional lymph node involvement is concerned, this hypothesis is reinforced by its specific association with distant relapse, i.e., in the lungs [37]. Moreover, there is also a concern that preoperative chemotherapy can result in a tumor response apparently sufficient to ablate perinephric infiltration and/or regional lymph node involvement but without completely eradicating microscopic residual disease, thus resulting in an inappropriate "downstaging" [38]. The case here reported confirmed the occurrence of marrow micrometastases detectable only before chemotherapy and disappearing afterward. For a minimally invasive evaluation of metastatic capability before preoperative chemotherapy, the bone marrow could thus represent a convenient site for sampling. Marrow involvement, although perhaps more transient than that of regional lymph nodes because of its probable higher sensitivity to anticancer drugs, is nevertheless more accessible than are nodes. Further investigation using sensitive techniques is warranted to determine the actual frequency of occult marrow micrometastases and whether such involvement is related to outcome. Bone marrow assessment at diagnosis might constitute a relapse-associated variable, useful in identifying a subset of patients with apparently localized disease but at high risk of recurrence.

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